such agents to the skin improves the appearance and psychological quality of life of patients whose lives have been dominated by severe psoriasis.

The present invention is further useful for peptide therapy. One example of peptide therapy is as a cytotoxic agent, for example as an antineoplastic agent. In this example, a bioconjugate containing the enzymatic domain of diphtheria toxin (DT) is administered to a subject such as described above for solid tumors or leukemia. The targeted release of the DT peptide results in the inhibition of protein synthesis and eventual cell death.

The present invention is also useful for gene therapy. One example of gene therapy is the delivery of an antisense oligonucleotide to inhibit viral gene expression and viral replication. In this example, a bioconjugate containing an antisense oligonucleotide against hepatitis B virus is administered to a patient having a hepatitis B infection. The accumulation of the bioconjugate and release of the antisense oligonucleotide in the liver inhibits hepatitis B virus gene expression and replication.

The present invention is further described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below were utilized.

## EXAMPLE 1

### Photolysis of $B_{12}$ and Co[SALEN] Bioconjugates

A protocol for a typical anaerobic continuous-wave photolysis of methylcob(III)alamin or $CH_3Cbl^{III}$ is as follows. An aqueous solution of 200 μM $CH_3Cbl^{III}$ and 50 mM $K^+$ Hepes pH 7.3 is placed in a 1 cm quartz cuvette. Samples that are to be photolyzed under anaerobic conditions are either subjected to repeated freeze-pump-thaw cycles, or purged with Ar for 40 minutes immediately prior to photolysis and sealed. Continuous-wave visible light irradiation is accomplished at the desired wavelength with an $Ar^+$ pumped dye laser. The incident light on the face of the cuvette is reduced to 12 mW $cm^{-2}$ with neutral density filters. The light flux is determined by potassium ferrioxalate actinometry and by a Scientech surface-reading thermopile. The cuvette is placed in a thermostated cell holder at 25-37°C. For quantum yield measurements as a function of magnetic field, the cuvette is placed in the gap of a GMW Associates

electromagnet with 7.5 cm diameter cylindrical poles. The magnetic field within the area of the cuvette is homogeneous to within 2% and the long term stability is better than 0.5% as monitored by a transverse Hall probe and digital teslameter.

Absorption spectra from 300-600 nm are recorded in one second with a diode array spectrophotometer at variable time intervals from 10 seconds to 2 minutes (depending upon the fluence of the photolyzing light source) for a total of 3 $\tau_{1/2}$. Exposure to light during analysis is kept to a minimum. The concentration of $CH_3Cbl^{III}$ is determined using the measured absorbance at 350 or 520 nm by the method of Chen and Chance (1993). The plot of $[CH_3Cbl^{III}]$ vs. time (t) appears zero-order in all cases.

Selection of Photolysis Wavelength: The absorption spectra for $CH_3Cbl^{III}$ and ethyl-Co[SALEN] are shown in Figures 1 and 2. For $CH_3Cbl^{III}$ the $\pi$-$\pi^*$ electronic transitions that lead to cleavage of the C-Co bond are maximal at 377 and 528 nm. Much preliminary work with $B_{12}$ photolysis has been carried out with 514 nm light from an Argon-ion ($Ar^+$) laser. This is close to the long-wavelength maximum absorbance and gives a quantum yield of about 0.3 for $CH_3Cbl^{III}$.

The absorbance of blood and tissue is significant at this optimal wavelength for cob(III)alamin excitation. Blood has a low (partial) transmittance window near 514 nm. This absorbance is sufficient to quickly pyrolize whole bovine blood placed in the light path of a 20 $W/cm^2$ beam of 514 nm light.

It would therefore be beneficial to provide a cobalamin for conjugation wherein the $\pi$-$\pi^*$ electronic transitions that lead to cleavage of the C-Co bond are maximal at a wavelength where there is minimal or no interference. Above about 610 nm blood becomes partially transparent and losses beyond 50% transmittance are largely due to light scattering from the erythrocytes. Heparinized bovine blood placed in the light path of a 20 $W/cm^2$ beam of 630 nm light shows only minor heating over long exposure times. There is also demonstrated a high transmittance of tissue at 610-800 nm.

This suggests the use of an organocobalt complex for conjugation having an absorption wavelength where tissue and blood are relatively transparent. Figure 2 shows that ethyl-Co[SALEN] complexes have absorption maximums near 650 nm, with significant absorption beyond 700 nm. An $Ar^+$-pumped dye laser or a Krypton-ion ($Kr^+$) laser can be a suitable high-intensity source of photons in the region of $610^+$ nm. $Ar^+$-pumped dye lasers are

often used for photodynamic therapy with hematoporphyrins. Also, an inexpensive He-Ne laser, having a principal line at 633 nm might be used. However, such lasers are typically limited to 50 mW maximum output.

There are laser dyes in the 600-700 nm region that can achieve energy conversion efficiencies of up to 45%. This means that a 6 W $Ar^+$ pump laser can yield nearly 3 W of spatially-coherent monochromatic light in the region of 610-750 nm. The exact wavelength can be chosen to optimize the continuous-wave quantum yield and still maintain a reasonable degree of tissue penetration. In tests with alkyl-Co[SALEN] complexes it has been found that 690 nm light from an $Ar^+$-pumped dye laser operating with rhodamine 6-G dye is satisfactory. Optimization can be determined depending on the specific cobalamin chosen for animal and/or clinical trials of the bioconjugates. In addition, high-power diode lasers that emit red light of the desirable wavelength are commercially available. These diode lasers have the advantage of providing up to 100 watts of optical power in a narrow region of the optical spectrum that is useful for triggering cleavage of the bioconjugates.

## EXAMPLE 2

### Sonolysis of $B_{12}$ and Co[SALEN] Bioconjugates

Sonolysis was carried out with a Branson ultrasonic bath (model 3200) operating at 47 kHz. The correct placement of the reaction vessel at a focal point of high- intensity ultrasound was determined by the oxidation of iodide to iodine in the presence of starch (Mason, 1991) and the temperature of the bath was maintained at 21° C by a constant temperature circulator. Aerobic sonolysis was typically carried out in a test tube or Erlenmeyer flask, whereas anaerobic sonolysis was carried out in a closed reaction vessel fitted with a sidearm and quartz cuvette. Anaerobic conditions were produced by sparging with Ar for 30 min prior to sonolysis. In some experiments, the pH was buffered by the use of 100 mM phosphate (aerobic experiments) or 100 mM *N*-(2-hydroxyethyl)piperazine-*N*-2-ethanesulfonate (Hepes) (anaerobic experiments), as specified. All procedures were carried out in the absence of light to prevent photolytic cleavage of the Co-C bond. Absorption spectra were recorded on a diode array spectrophotometer (HP 8452A). The solutions were transferred to a quartz cuvette with a 1 cm light path for all optical measurements and care was exercised to ensure that insignificant photolysis occured during the 1 s measurement time.